REMARKS

Please reconsider the application in view of the following remarks. Applicant thanks the Examiner for carefully considering this application.

Disposition of the Claims

Claims 1, 5-7, and 22-25 are pending. Claim 1 is independent. The remaining claims depend, directly or indirectly, from claim 1.

Rejections under 35 U.S.C. § 112

Claims 1, 5-7, and 22-25

Claims 1 and 5-7 are rejected under 35 U.S.C. 112, second paragraph, as being failing to comply with the written description requirements. Specifically, the Examiner assets that only reaction with benzyl group under a condition of pH 5-6 is disclosed, but no description is found for allyl or diphenylmethyl group. This rejection is respectfully traversed.

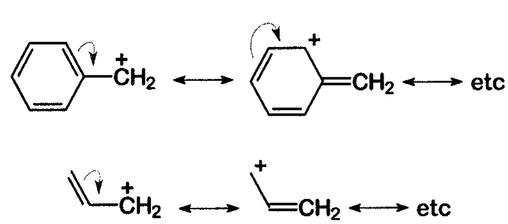
The test for determining compliance with the written description requirement is whether the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter, rather than the presence or absence of literal support in the specification for the claim language. *In re Herschler*, 591 F.2d 693, 700 (CCPA 1979); *In re Edwards*, 568 F.2d 1349, 1351-52 (CCPA 1978).

In addition, the specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir.

1991); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).

Reactions between alkyl halides and carboxylic acid salts to form esters are well-known in the art. *See e.g.*, H.E. Hennis et al., "*Esters from Reactions of Alkyl Halides and Salts of Carboxylic Acids. Reactions of Primary Alkyl Chlorides and Sodium Salts of Carboxylic Acids,*" Ind. Eng. Chem. Prod. Res. Dev., 6(3), pp. 193-195 (1967), **http://pubs.acs.org/doi/abs/10.1021/i360026a003**. In this reaction, the carboxylic salt acts as a nucleophile displacing the halide leaving group on the alkyl halide to form an ester.

Similar reactions can take place between carboxylic acid salts and allyl or benzyl halides. In these similar reactions, it is well-known that allyl halides and benzyl halides are significantly more reactive than the corresponding primary alkyl halides (e.g., ethyl halides or propyl halides). *See e.g.*, **http://pages.towson.edu/ryzhkov/handouts/sehand.html** or **http://www.wwu.edu/pavia/chap10c.ppt**. The allyl or benzyl halides are more reactive than the alkyl halides in such substitution reactions because the double bonds at the β and γ positions can stabilize the intermediates, which have cationic characteristics, by resonance (see below). The resonance structures lower the transition state activation energy barriers.



For example, the relative reaction rates for the alkyl chloride, allyl chloride, and benzyl chloride are shown in the following table (from the Western Washington University PPT slides noted above). The reaction rates of ally chloride and benzyl chloride are at least 100 times faster than that of ethyl chloride, while the reaction rates of allyl chloride and benzyl chloride are comparable (within a factor of two).

Halides	**Rel. Reaction Rates**
Ethyl chloride	Very small
Isopropyl chloride	1.0
Allyl chloride	74
Benzyl chloride	140

The present specification teaches that, to prevent the sialic acids from being cut off by an acid, the carboxyl group of a sialic acid may be protected by a protective group, such as benzyl, allyl, or diphenylmethyl. (Paragraph [0125]). The pKa values of carboxyl groups of sialic acids are less than 5. Therefore, the carboxyl group of a sialic acid would be deprotonated (i.e., forming a salt) at pH 5 to 6. The ionized carboxylate is a nucleophile, which can react with an allyl or benzyl halide to form an ester, as noted above. A specific example, using benzyl bromide at pH 5-6, is described in the specification. Because allyl halides and benzyl halides are known to have similar reactivities in such ester formation reactions, one skilled in the art would know that similar reactions using allyl halides and diphenylmethyl halides (which are substituted benzyl halides) would occur under similar conditions.

Because allyl halides and diphenylmethyl halides are known to have similar reactivities as benzyl halides, additional reaction details using allyl halides or diphenylmethyl

halides need not be described, and preferably be omitted. Accordingly, withdrawal of this rejection is respectfully requested.

Rejections under 35 U.S.C. § 103(a)

Claims 1, 5-7, and 22-25

Claims 1, 5-7, and 22-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Meinojohanns (J. Chem. Soc. Perkin Trans. 1, 1998, pages 549-560, PTO-1449 submitted December 20, 2007) (hereinafter "Meinojohanns") in view of Keil, et al. (Angew. Chem. Int. Ed., 40, No. 2 (2001), pp. 366-369) (hereinafter "Keil") and Greene, et al. (Protective Groups in Organic Synthesis, Third Edition, John Wiley & Sons, Inc. (1999); pp. 415-419) (hereinafter "Greene"). This rejection is respectfully traversed.

The present invention relates to processes of preparing a glycopeptides having an asparagine-linked oligosaccharide at a desired position of the peptide chain. Specifically, claim 1 requires, *inter alia*, "preparing an asparagine-linked disialooligosaccharide or an asparagine-linked monosialooligosaccharide having amino group nitrogen protected with a fat-soluble protective group and the carboxyl group of the sialic acid protected with a benzyl, allyl, or diphenylmethyl group, wherein the benzyl, allyl, or diphenylmethyl group is introduced into the carboxyl group of the sialic acid under a condition of pH 5 to 6."

As acknowledged by the Examiner, Meinojohanns does not utilize an asparagine building block which contains a sialic acid moiety, but instead suggests the uses of sialyltransferase to introduce sialyl moieties into the product. (Office Action, p. 5, last line – p.

6, line 2). The Examiner relies on Keil for teaching the preparation of a sialic acid containing compound 20, which is prepared using a building block 7. (Office Action, p. 6).

The building block 7 in Keil is a threonine glycoside containing a monosaccharide and a sialyl group. First, this is an O-linked glycoside, not an N-linked glycoside. More importantly, one skilled in the art would appreciate that such solution synthesis would be impractical for an oligosaccharide (instead of a monosaccharide) intermediate.

The Examiner further relies on Greene for teaching protection of a carboxylic acid using a benzyl alcohol. Applicant respectfully notes that Greene's ester formation method (i.e., acid-catalyzed dehydration reactions) cannot be used to protect carboxyl groups of sialic acids on oligosaccharides.

As shown in the attached article by Y. Kajihara et al., "*Chemoenzymatic Synthesis of Diverse Asparagine-Linked Oligosaccharides*," in Methods in Enzymology, Vol. 362, Elsevier (USA) 2003, acid-catalyzed reactions would result in the removal of sialic acid from oligosacccharides. See for example, the synthesis of asialononasaccharide 5 on page 53:

> "To a solution of Asn-disialyloligosaccharide 2 (98 mg, 42 μmol) in water (5.8 ml) is added HCl solution (2 M, 100 μl) and this mixture is stirred at 80°. After 2 h, the mixture is cooled to 4° and neutralized with aqueous $NaHCO_3$. This solution is then lyophilized. Purification of the residue by gel-permeation column (Sephadex G-25, ϕ2.5×100 cm; water) affords asialooligosaccharide 5 (67 mg, 91%)."

Similarly, in Example 2 in U.S. Patent No. 7,135,566 (Col. 34), the inventor showed that when a sialyloligosaccharide-asparagine compound (e.g., compound 24) is treated with acid, de-sialyl compounds (e.g., compounds 25, 29, and 33) are formed.

Therefore, one skilled in the art would not be motivated to use Greene's method to protect the sialic acids on oligosaccharides.

In contrast to Greene's methods, the methods for protecting carboxylic acids of sialic acids on oligosaccharides disclosed in this application produce unexpected good results. These methods produce protected sialooligosaccharides in good yields without desialylation.

Therefore, claim 1 is patentable over Meinojohanns in view of Keil and Greene. Dependent claims 5-7 and 22-25 should also be patentable for at least the same reasons. Accordingly, withdrawal of this rejection is respectfully requested.

Conclusion

Applicant believes this reply is fully responsive to all outstanding issues and places this application in condition for allowance. If this belief is incorrect, or other issues arise, the Examiner is encouraged to contact the undersigned or his associates at the telephone number listed below. Please apply any charges not covered, or any credits, to Deposit Account 50-0591, Reference 17563/003001.

Dated: October 13, 2010

Respectfully submitted,

By T. Chyau Liang

T. Chyau Liang, Ph.D.
Registration No.: 48,885
OSHA · LIANG LLP
Two Houston Center
909 Fannin Street, Suite 3500
Houston, Texas 77010
(713) 228-8600
(713) 228-8778 (Fax)
Attorney for Applicant

Attachment: Kajihara – Methods in Enzymology

[5] Chemoenzymatic Synthesis of Diverse Asparagine-Linked Oligosaccharides

By Yasuhiro Kajihara, Yasuhiro Suzuki, Ken Sasaki, and Lekh Raj Juneja

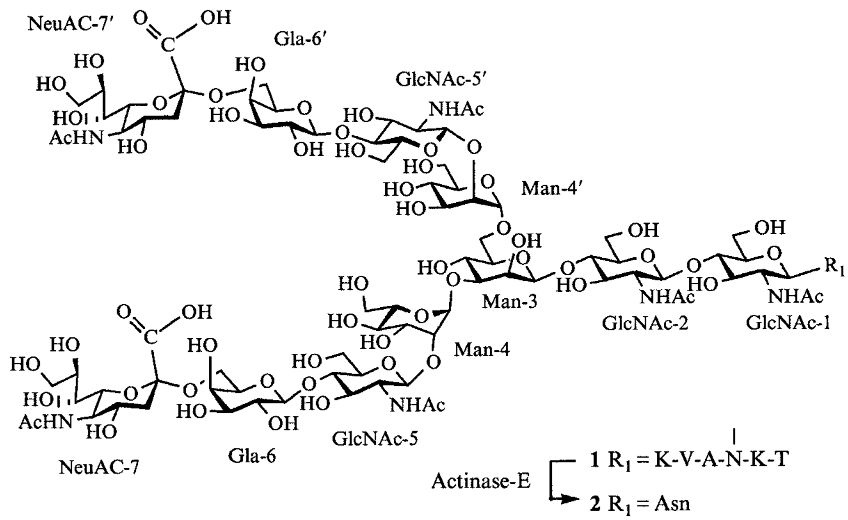
Introduction

Most oligosaccharides on proteins are divided into *O*-glycans and *N*-glycans. To investigate the roles of the oligosaccharides, chemical and chemoenzymatic syntheses of these oligosaccharides have been performed.[1–3] Technologies for the synthesis of large oligosaccharides have advanced considerably; however, chemical synthesis is time consuming because of repetitive protection/deprotection steps. *N*-Glycans in glycoproteins show structural diversity in their oligosaccharides, called glycoforms. To study why glycoproteins show different glycoforms, *N*-glycans having diverse structures must be prepared and are used for bioassay. Semisynthetic methods have also been developed.[4–7] Using these approaches[4–6] oligosaccharides from natural sources such as commercially available glycoproteins can yield large quantities of pure oligosaccharide.

We have used asparagine-linked biantennary complex-type sialylundecasaccharide **1**[8] (see Scheme 1) obtained from egg yolk to prepare more than 20 kinds of a pure asparagine-linked oligosaccharide (Asn-oligosaccharide), using branch-specific glycosidase digestion. In short, monosialylo-ligosaccharides are prepared from **2** by acid hydrolysis of NeuAc, and subsequent exoglycosidase digestion (β-galactosidase, *N*-acetyl-β-D-glucosaminidase, and α-D-mannosidase) of the individual asialo branch affords corresponding diverse oligosaccharides.

[1] K. Toshima and K. Tatsuta, *Chem. Rev.* **93,** 1503 (1993).
[2] C. H. Wong, R. L. Halcomb, Y. Ichikawa, and T. Kajimoto, *Angew. Chem. Int. Ed.* **34,** 521 (1995).
[3] C. Unverzagt, *Carbohydr. Res.* **305,** 423 (1998).
[4] T. Tamura, M. S. Wadhwa, and K. G. Rice, *Anal. Biochem.* **216,** 335 (1994).
[5] K. G. Rice, P. Wu, L. Brand, and Y. C. Lee, *Biochemistry* **32,** 7264 (1993).
[6] E. Meinjohanns, M. Meldal, H. Paulsen, R. A. Dwek, and K. J. Bock, *J. Chem. Soc. Perkin Trans.* **1,** 549 (1998).
[7] C. H. Lin, M. Shimazaki, C. H. Wong, M. Koketsu, L. R. Juneja, and M. Kim, *Bioorg. Med. Chem.* **3,** 1625 (1995).
[8] A. Seko, M. Koketsu, M. Nishizono, Y. Enoki, H. R. Ibrahim, L. R. Juneja, M. Kim, and T. Yamamoto, *Biochim. Biophys. Acta* **1335,** 23 (1997).

0076-6879/03 $35.00

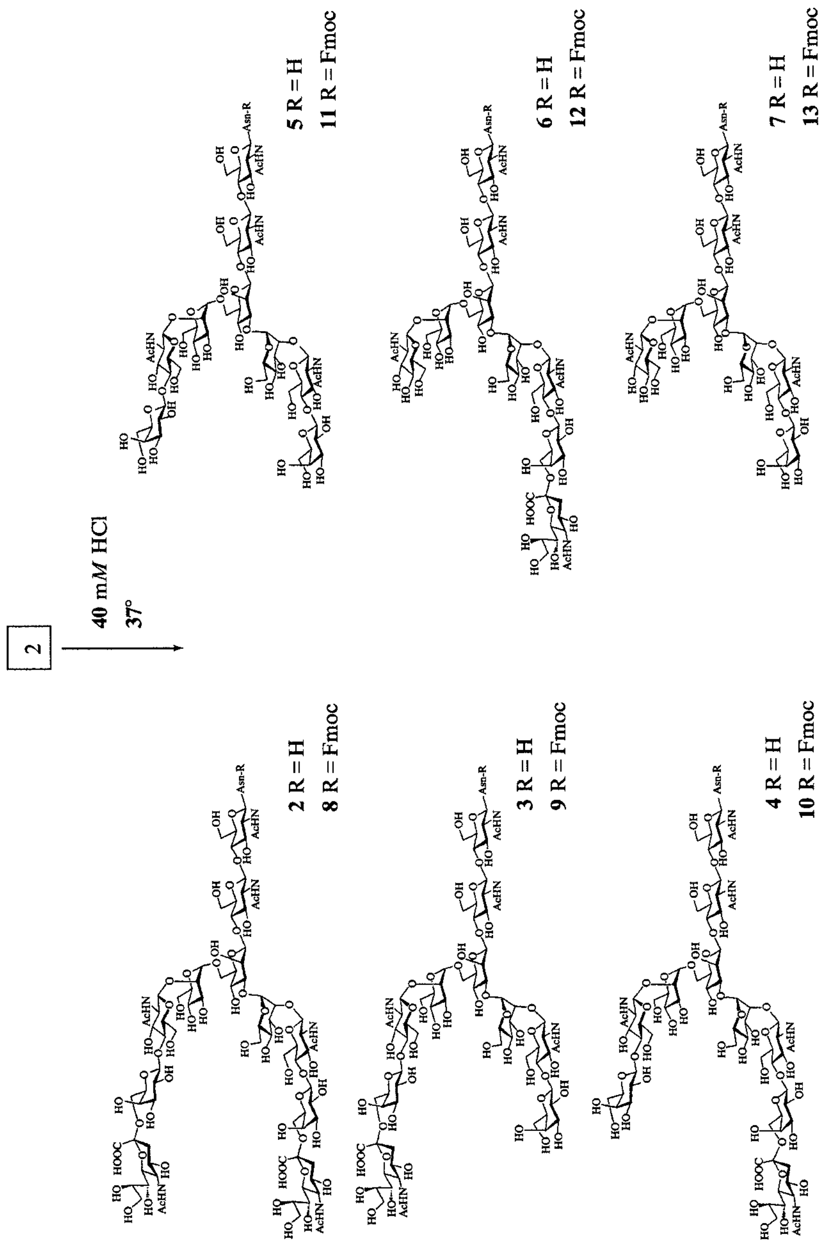


SCHEME 1

Synthesis of Asparagine-Linked Oligosaccharide Derivatives

Asn-oligosaccharide **2** is prepared by protease (actinase E) digestion of **1**. To release one of the two NeuAc residues from **2**, acid hydrolysis of Asn-oligosaccharide **2** by 40 m*M* HCl solution is performed. This reaction affords four kinds of Asn-oligosaccharide, **2**, **3**, **4**, and **5**, along with **6** and **7** as contaminants. To obtain pure monosialyloligosaccharides **3** and **4**, purification by high-performance liquid chromatography (HPLC) (ODS column) was considered. However, because these oligosaccharides are hydrophilic, purification could not be attained in milligram scale. Therefore, oligosaccharides **2–7** are protected by a hydrophobic protecting group such as 9-fluorenylmethyl group (Fmoc) in order to increase their hydrophobicity (Scheme 2; compounds **2–13**). As expected, this increases interaction between Fmoc-oligosaccharides **8–13** and the ODS column and affords each Asn-oligosaccharide (Fig. 1A) except **9** and **10**. The mixture of **9** and **10** is further protected by forming a benzyl ester of NeuAc. This treatment enables us to purify monosialyloligosaccharides **14** and **15** (Scheme 3; compounds **14–17**).

Galactosidase digestion of **9** and **10** affords nonasaccharides **12** and **16**. The limit of this purification is about 20 mg in one purification step by HPLC [ODS; inner diameter (ϕ) 20×250 mm]. Repeat chromatography on the same column yields 200 mg of each monosialyloligosaccharide, **12** and **16** (Scheme 3 and Fig. 1B). Each monosialyloligosaccharide, **12** and


2
40 m*M* HCl
37°
2 R = H
8 R = Fmoc
3 R = H
9 R = Fmoc
4 R = H
10 R = Fmoc
5 R = H
11 R = Fmoc
6 R = H
12 R = Fmoc
7 R = H
13 R = Fmoc

SCHEME 2

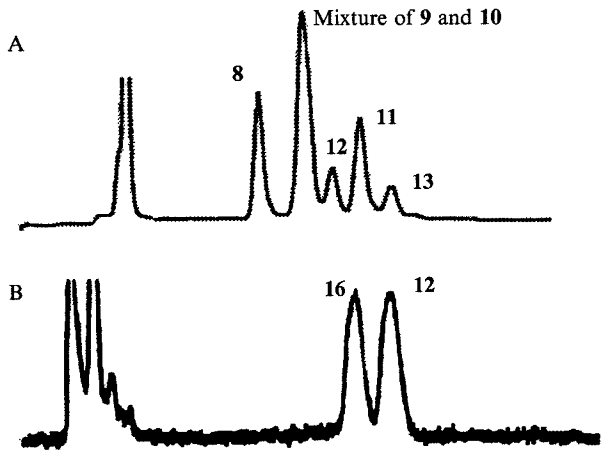


FIG. 1. HPLC profile of (A) acid hydrolysis reaction and (B) purification of **12** and **16** after galactosidase digestion of **9** and **10**.

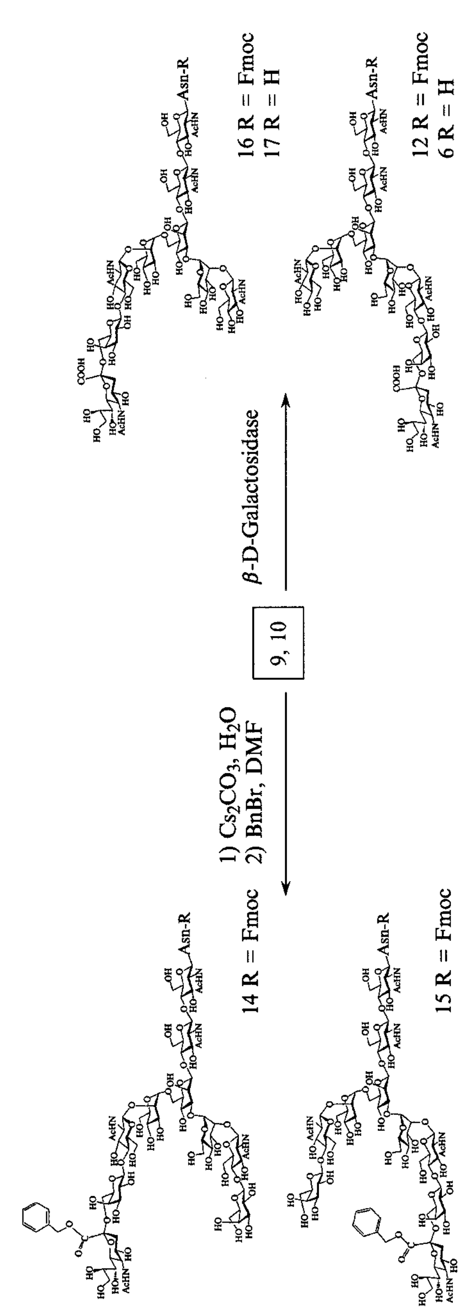
16, is separately treated by exoglycosidase digestion as shown in Schemes 4 (compounds **18–27**) and 5 (compounds **28–37**). Each exoglycosidase digestion can be performed on a 100-mg scale and yield ranges from 70 to 90% (isolated yield). In addition, simultaneous treatment of several exoglycosidase digestions can also be performed as shown in Scheme 6 (compounds **38** and **39**). Asn-oligosaccharides, shown in Fig. 2 (compounds **40–51**), can also be prepared from **12** by the same strategy shown in Schemes 2–5. The structure of these oligosaccharides thus obtained can be determined by use of a reporter group[9] and high-resolution mass spectroscopy. The nuclear magnetic resonance (NMR) data for these oligosaccharides identifies the product. Removal of the *N*-9-fluorenylmethyl group is performed with morpholine in dimethylformaside (DMF) solution in moderate yield. Using this strategy, more than 24 kinds of Asn-oligosaccharides are obtained as shown in Schemes 2–5 and Fig. 2.

Procedures

General Methods

Crude sialylglycopeptide **1** is prepared from egg yolk by the reported method[8] of extraction with aqueous phenol and simple gel permeation

[9] J. F. G. Vliegenthart, L. Dorland, and H. Halbeek, *Adv. Carbohydr. Chem. Biochem.* **41,** 209 (1983).



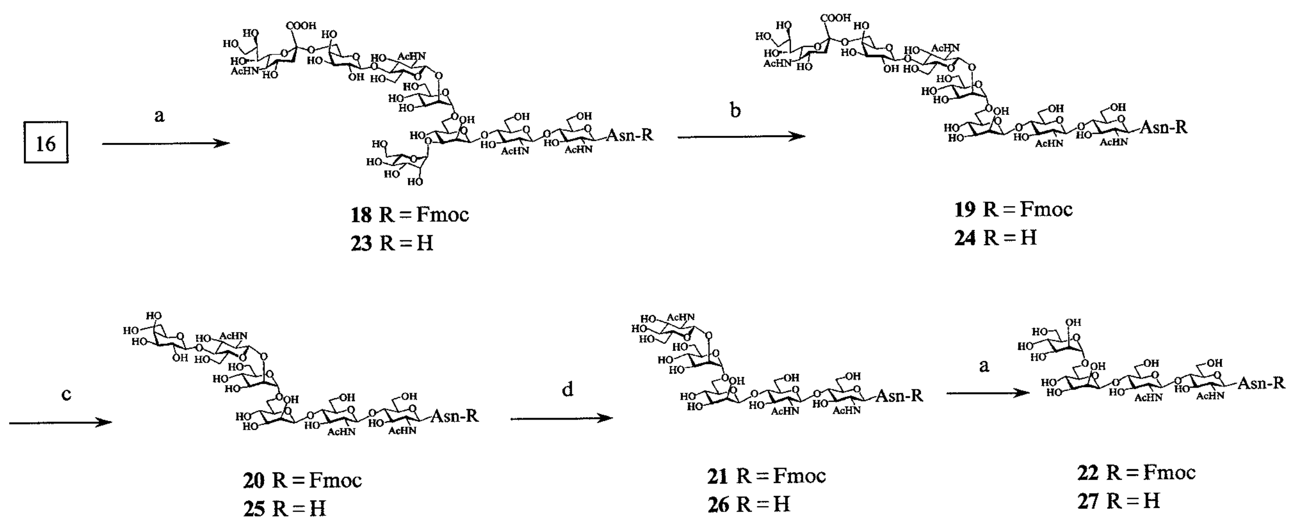
14 R = Fmoc

15 R = Fmoc

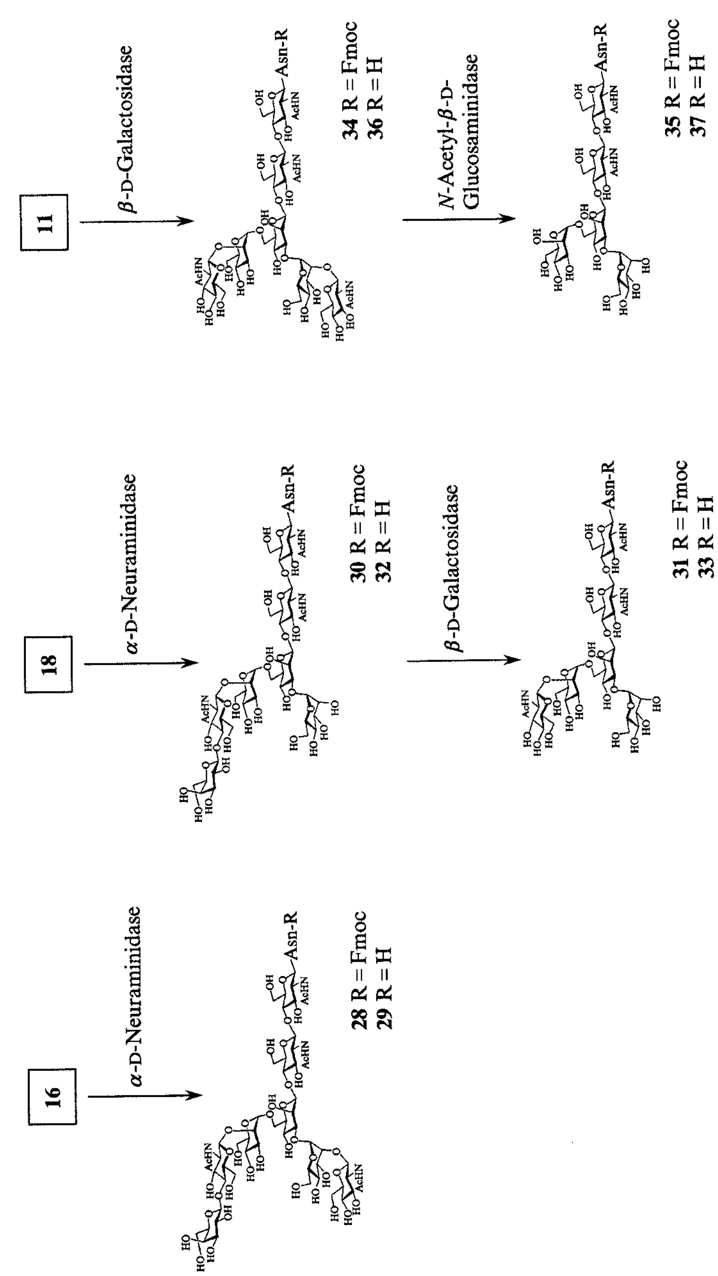
16 R = Fmoc
17 R = H

12 R = Fmoc
6 R = H

SCHEME 3

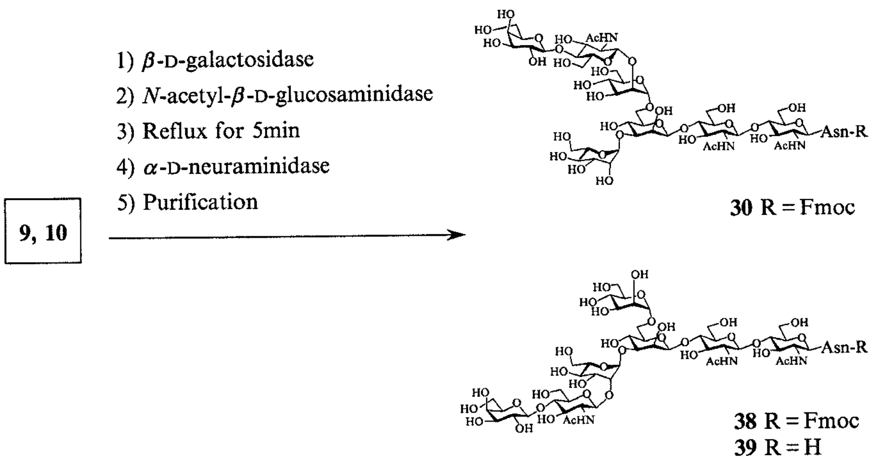


SCHEME 4. (a) *N*-Acetyl-β-D-glucosaminidase; (b) α-D-mannosidase; (c) α-D-neuraminidase; (d) β-D-galactosidase.



11
β-D-Galactosidase
34 R = Fmoc
36 R = H
N-Acetyl-β-D-Glucosaminidase
35 R = Fmoc
37 R = H
18
α-D-Neuraminidase
30 R = Fmoc
32 R = H
β-D-Galactosidase
31 R = Fmoc
33 R = H
16
α-D-Neuraminidase
28 R = Fmoc
29 R = H

SCHEME 5



30 R = Fmoc

38 R = Fmoc
39 R = H

SCHEME 6

(Sephadex G-25) and is about 50% pure. Actinase E is purchased from Kaken Pharmaceutical (Osaka, Japan). Jack bean β-D-galactosidase is purchased from Seikagakukogyo (Japan). Jack beans *N*-acetyl-β-D-glucosaminidase, jack bean β-D-mannosidase, and *Vibrio cholerae* α-D-neuraminidase are purchased from Sigma (St. Louis, MO). NMR spectra are measured with a Bruker BioSpin (Billerica, MA) Avance 400 [30°, internal standard HOD = 4.718 ppm; external (or internal) standard acetone = 2.225 ppm][9] instrument.

Synthesis of Compound **2**

To a solution of crude sialylglycopeptide **1** (809 mg) and NaN_3 in Tris-HCl buffer (50 m*M*; with 10 m*M* $CaCl_2$, pH 7.5; 32 ml) is added actinase E (263 mg, the same Tris-HCl buffer, 8 ml) and this mixture is incubated at 37° for 60 h. During incubation, the pH is kept at pH 7.5. After 60 h, additional actinase E (25 mg) is added and the mixture is incubated for a further 55 h. This reaction is monitored by thin-layer chromatography (TLC) [1 *M* ammonium acetatc–2-propanol, 1:1 (v/v)]. After the reaction, the mixture is lyophilized and purified by gel permeation (Sephadex G-25; ϕ 2.5 × 100 cm; H_2O) to afford Asn-sialyloligosaccharide **2**[10] (301 mg).

^1H NMR δ 5.13 (s, 1H, Man4-H-1), 5.07 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.95 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.60 (m,

[10] M. Mizuno, K. Haneda, R. Iguchi, I. Muramoto, T. Kawakami, S. Aimoto, K. Yamamoto, and T. Inazu, *J. Am. Chem. Soc.* **121,** 284 (1999).

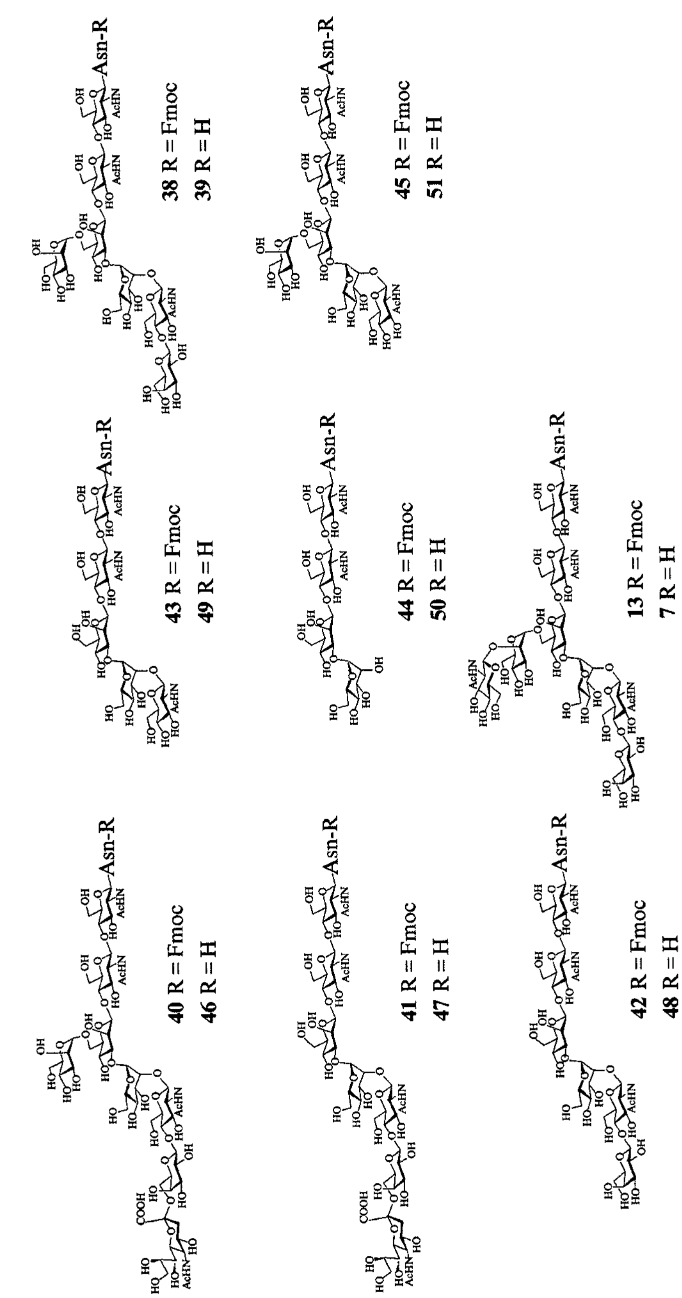


FIG. 2. Structure of oligosaccharide derivatives.

3H, GlcNAc2,5,5′-H-1), 4.44 (d, 2H, $J = 8.0$ Hz, Gal6,6′-H-1), 4.25 (bs, 1H, Man3-H-2), 4.20 (bd, 1H, Man4-H-2), 4.12 (bd, 1H, Man4′-H-2), 2.67 (bdd, 2H, $J = 4.6$ Hz, 12.4 Hz, NeuAc7,7′-H-3eq), 2.16 (s, 3H, Ac), 2.15 (s, 6H, Ac × 2), 2.02 (s, 6H, Ac × 2), 1.71 (dd, 2H, $J = 12.4$ Hz, 12.4 Hz, NeuAc7,7′-H-3ax).

*Asialononasaccharide **5***

To a solution of Asn-disialyloligosaccharide **2** (98 mg, 42 μmol) in water (5.8 ml) is added HCl solution (2 *M*, 100 μl) and this mixture is stirred at 80°. After 2 h, the mixture is cooled to 4° and neutralized with aqueous $NaHCO_3$. This solution is then lyophilized. Purification of the residue by gel-permeation column (Sephadex G-25, ϕ 2.5 × 100 cm; water) affords asialooligosaccharide **5**[6] (67 mg, 91%).

^{1}H NMR δ 5.12 (s, 1H, Man4-H-1), 5.07 (d, 1H, $J = 9.7$ Hz, GlcNAcl-H-1), 4.92 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.62 (d, 1H, $J = 8.0$ Hz, GlcNAc2-H-1), 4.58 (d, 2H, $J = 7.8$ Hz, GlcNAc5,5′-H-1), 4.47 (d, 2H, $J = 7.9$ Hz, Gal6,6′-H-1), 4.24 (bd, 1H, Man3-H-2), 4.19 (bdd, 1H, $J = 3.2$ Hz, 1.4 Hz, Man4-H-2), 4.12 (bdd, 1H, $J = 3.2$ Hz, 1.4 Hz, Man4′-H-2), 2.08 (s, 3H, Ac), 2.05 (s, 6H, Ac × 2), 2.01 (s, 3H, Ac).

*Fmoc-oligosaccharides **8**, **9**, **10**, and **11***

To a solution of Asn-oligosaccharide **2**, (1.07 g, 456 μmol) in water (11.4 ml) is added HCl solution (80 m*M*, 11.4 ml) and this mixture is stirred at 37°. After 6 h, the mixture is cooled to 4° and neutralized with aqueous $NaHCO_3$, and lyophilized. Purification of the residue by gel-permeation column (Sephadex G-25, ϕ 2.5 × 100 cm; water) affords a mixture (778 mg) of disialo substrate **2**, monosialyloligosaccharides **3** and **4**, and asialooligosaccharide **5**, along with **6**, and **7**. This mixture is then used for the next protecting reaction. To a solution of this mixture (778 mg) in H_2O–acetone (3.8 ml–5.7 ml) is added $NaHCO_3$ (162 mg, 1.93 mmol) and 9-fluorenylmethyl-*N*-succimidylcarbonate (432 mg, 1.28 mmol), and the mixture is stirred at room temperature. After 2 h, the mixture is evaporated to remove acetone and desalted on an ODS column (ϕ 20 × 250 mm; eluted with H_2O, 100 ml, and then with 25% CH_3CN, 200 ml) to yield mixture of Fmoc-oligosaccharides, **8–13** (681 mg). This mixture is further purified by HPLC on an ODS column [YMC packed column D-ODS-5 S-5 120A, ϕ 20 × 250 mm; 50 m*M* ammonium acetate: CH_3CN, 82:18 (v/v); 3.5 ml/min; monitoring at 215 nm) to obtain disialyloligosaccharide **8** (retention time, 86 min), a mixture of monosialyloligosaccharides **9** and **10** (100 min), and asialooligosaccharide **11** (127 min). In addition, monosialylnonasaccharide **12** (112 min) and

asialooctasaccharide **13** (139 min) are also obtained. This purification is performed repeatedly with about 30 mg of mixture each time (total, about 500 mg). The individual oligosaccharides thus obtained are combined, lyophilized, and then desalted using HPLC (ODS column, ϕ 5 × 150 mm; eluted with H_2O, 100 ml, and then with 25% CH_3CN, 200 ml) to yield pure oligosaccharide **8** (148 mg, 13%), a mixture of **9** and **10** (249 mg, 24%), **11** (101 mg, 11%), **12** (68 mg, 7%), and **13** (35 mg, 4%).

*Disialooligosaccharide **8***

^{1}H NMR δ 7.92 (d, 2H, $J=7.5$ Hz, Fmoc), 7.72 (d, 2H, $J=7.5$ Hz, Fmoc), 7.51 (dd, 2H, $J=7.5$ Hz, Fmoc), 7.44 (dd, 2H, $J=7.5$ Hz, Fmoc), 5.14 (s, 1H, Man4-H-1), 5.00 (d, 1H, J = 9.4 Hz, GlcNAc1-H-1), 4.95 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.60 (m, GlcNAc2,5,5′-H-1), 4.45 (d, 2H, $J=7.8$ Hz, Gal6,6′-H-1), 4.35 (1H, Fmoc), 4.25 (bd, 1H, Man3-H-2), 4.20 (bdd, 1H, Man4-H-2), 4.11 (bd, 1H, Man4′-H-2), 2.73 (bdd, 1H, $J=1.7$ Hz, Asn-βCH), 2.52 (bdd, 1H, Asn-βCH), 2.67 (dd, 2H, $J=4.8$ Hz, 12.5 Hz, NeuAc7,7′-H-3eq), 2.07 (s, 9H, Ac × 3), 2.03 (s, 6H, Ac × 2), 1.89 (s, 3H, Ac), 1.72 (dd, 2H, $J=12.1$ Hz, 12.1 Hz, NeuAc7,7′-H-3ax).

*Asialooligosaccharide **11***

^{1}H NMR δ 7.91 (d, 2H, $J=7.5$ Hz, Fmoc), 7.70 (d, 2H, $J=7.5$ Hz, Fmoc), 7.50 (dd, 2H, $J=7.5$ Hz, Fmoc), 7.43 (dd, 2H, $J=7.5$ Hz, Fmoc), 5.12 (s, 1H, Man4-H-1), 5.00 (d, 1H, $J=9.4$ Hz, GlcNAc1-H-1), 4.93 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.58 (d, 3H, GlcNAc2,5,5′-H-1), 4.47 (d, 2H, $J=7.9$ Hz, Gal6,6′-H-1), 4.33 (1H, Fmoc), 4.24 (bd, 1H, $J=2.1$ Hz, Man3-H-2), 4.19 (bd, 1H, $J=2.7$HZ, Man4-H-2), 4.11 (bd, 1H, $J=3.0$ Hz, Man4′-H-2), 2.72 (bdd, 1H, $J=1.7$ Hz, 15.4 Hz, Asn-βCH), 2.52 (bdd, 1H, $J=9.2$ Hz, 15.4 Hz, Asn-βCH), 2.07 (s, 3H, Ac), 2.05 (s, 6H, Ac × 2), 1.89 (s, 3H, Ac).

*Monosialyloligosaccharide **12***

^{1}H NMR δ 7.92 (d, 2H, $J=7.5$ Hz, Fmoc), 7.71 (d, 2H, $J=7.5$ Hz, Fmoc), 7.51 (dd, 2H, $J=7.5$ Hz, Fmoc), 7.43 (dd, 2H, $J=7.5$ Hz, Fmoc), 5.14 (s, 1H, Man4-H-1), 5.00 (d, 1H, GlcNAc1-H-1), 4.92 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.61 (d, 1H, $J=8.0$ Hz GlcNAc2-H-1), 4.56 (d, 2H, GlcNAc5,5′-H-1), 4.45 (d, 1H, $J=7.8$ Hz, Gal6-H-1), 4.35 (bt, 1H, Fmoc), 4.25 (bd, 1H, Man3-H-2), 4.20 (bd, 1H, Man4-H-2), 4.11 (bd, 1H, Man4′-H-2), 2.72 (bdd, 1H, Asn-βCH), 2.67 (dd, 1H, $J=4.3$ Hz, 12.4 Hz, NeuAc7-H-3eq), 2.54 (bdd, 1H, $J=9.2$ Hz, 15.9 Hz, Asn-βCH),

2.07 (s, 6H, Ac × 2), 2.05, 2.03, 1.89 (each s, each 3H, Ac), 1.72 (dd, 1H, $J = 12.1$ Hz, 12.1 Hz, NeuAc7-H-3ax).

Asialooctasaccharide ***13***

^{1}H NMR δ 7.92 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.72 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.51 (dd, 2H, $J = 7.5$ Hz, Fmoc), 7.44 (dd, 2H, $J = 7.5$ Hz, Fmoc), 5.12 (s, 1H, Man4-H-1), 5.00 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.92 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.57 (bd, 1H, GlcNAc2-H-1), 4.55 (d, 2H, $J = 8.9$ Hz, GlcNAc5,5′-H-1), 4.46 (dd, $J = 7.8$ Hz, Gal6-H-1), 4.35 (t, 1H, Fmoc), 4.24 (bd, 1H, Man3-H-2), 4.19 (bd, 1H, Man4-H-2), 4.11 (bd, 1H, Man4′-H-2), 2.72 (1H, bdd, $J = 15.4$ Hz, Asn-βCH), 2.51 (bdd, 1H, $J = 9.6$ Hz, 13.8 Hz, Asn-βCH), 2.06 (s, 3H, Ac), 2.05 (s, 6H, Ac × 2), 1.89 (s, 3H, Ac).

Benzyl Ester Monosialyloligosaccharides ***14*** *and* ***15***

A solution of a mixture of Fmoc-monosialyldecasaccharides **9** and **10** (5.0 mg) in cold H_2O (1 ml, 4°) is passed through to a column [ϕ 0.5 cm × 5 cm containing Dowex 50W × 8(H^+) resin], and the column is washed with 10 ml of cold water. The eluant and the washing are pooled and lyophilized. This residue is dissolved in H_2O (0.22 ml) and neutralized (pH 7) by stepwise addition of a solution of Cs_2CO_3 (2.5 mg/ml) while monitoring with a pH meter, and lyophilized. The residue is dissolved in dry DMF (0.43 ml) and is mixed with a solution of benzyl promide (BnBr, 6.6 μl) in DMF (20 μl) and stirred at room temperature under an argon atmosphere. After 48 h, diethyl ether (5 ml) is added and the precipitate formed is collected. Purification of the precipitated material by HPLC [ODS column, ϕ 20 × 250 mm; ammonium acetate–CH_3CN, 78:22 (v/v)] affords monobenzylsialyloligosaccharides **14** (91 min) and **15** (88 min). Desalting of each product by HPLC (ODS column, ϕ 5 × 150 mm; H_2O, 50 ml, and then 25% CH_3CN, 100 ml) affords pure monobenzylsialyloligosaccharides, **14** (1.6 mg) and **15** (1.8 mg).

Compound ***14***

^{1}H NMR δ 7.91 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.71 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.53–7.41 (m, 9H, Fmoc, -CH_2-Ph), 5.38 (d, 1H, $J = 12.1$ Hz, -CH_2-Ph), 5.31 (d, 1H, $J = 12.1$ Hz, -CH_2-Ph), 5.12 (s, 1H, Man4-H-1), 4.99 (d, 1H, $J = 9.8$ Hz, GlcNAc1-H-1), 4.93 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.58 (m, 3H, GlcNAc2,5,5′-H-1), 4.46 (1H, d, $J = 7.8$ Hz, Gal6-H-1), 4.33 (d, 1H, $J = 7.8$ Hz, Gal6′-H-1), 4.24 (bs, 1H, Man3-H-2), 4.19 (bs, 1H, Man4-H-2), 4.11 (bs, 1H, Man4′-H-2), 2.72 (bd, 1H, Asn-βCH), 2.68

(dd, 1H, $J = 4.8$ Hz, 13.0 Hz, NeuAc7-H-3eq), 2.52 (bdd, 1H, $J = 9.7$ Hz, 14.1 Hz, Asn-βCH), 2.06, 2.05, 2.04, 2.02, 1.89 (each s, each 3H, Ac), 1.84 (dd, 1H, $J = 13.0$ Hz, 13.0 Hz, NeuAc7-H-3ax).

*Compound **15***

^{1}H NMR δ 7.91 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.71 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.53–7.41 (m, 9H, Fmoc, -CH_2-Ph), 5.38 (d, 1H, $J = 12.1$ Hz, -CH_2-Ph), 5.31 (d, 1H, $J = 12.1$ Hz, -CH_2-Ph), 5.12 (s, 1H, Man4-H-1), 4.99 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.92 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.58 (m, 3H, GlcNAc2,5,5′-H-1), 4.47 (d, 1H, $J = 7.9$ Hz, Gal6′-H-1), 4.33 (d, 1H, $J = 7.9$ Hz, Gal6-H-1), 4.24 (bs, 1H, Man3-H-2), 4.19 (bs, 1H, Man4-H-2), 4.11 (bs, 1H, Man4′-H-2), 2.72 (bd, 1H, Asn-βCH), 2.68 (dd, 1H, $J = 4.6$ Hz, 12.7 Hz, NeuAc7-H-3eq), 2.52 (dd, 1H, J = 8.7 Hz, 15.0 Hz, Asn-βCH), 2.06, 2.05, 2.04, 2.02, 1.89 (each s, each 3H, Ac) 1.84 (dd, 1H, $J = 12.7$ Hz, 12.7 Hz, NeuAc7-H3ax).

*Galactosidase Digestion of Monosialyldecasaccharides **9** and **10***

To a mixture of monosialyldecasaccharides **9** and **10** (135 mg, 59.4 μmol) in HEPES buffer (50 m*M*, pH 6.0, 5.6 ml) containing bovine serum albumin (1.0 mg) is added β-galactosidase (390 mU in 50 m*M* HEPES buffer, 100 μl, pH 6.0) and this mixture is incubated at 37° for 19 h, and lyophilized. Purification of the residue by HPLC [YMC packed column D-ODS-5 S-5 120A, ϕ 20 × 250 mm; 50 m*M* ammonium acetate–CH_3CN, 82:18 (v/v); 3.5 ml/min] affords monosialyloligosaccharides **16** (170 min) and **12** (182 min). This purification is performed repeatedly with ~10 to 20-mg portions of the reaction mixture (total, ~120 mg). Desalting of each product by HPLC (ODS column; ϕ 5 × 150 mm; H_2O, 100 ml and then 25% CH_3CN solution, 200 ml) yields pure oligosaccharides **16** (51 mg, 41%) and **12** (60 mg, 48%).

*Monosialylnonasaccharide **16***

^{1}H NMR δ 7.92 (d, 2H, Fmoc), 7.71 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.51 (dd, 2H, $J = 7.5$ Hz, Fmoc), 7.44 (dd, 2H, $J = 7.5$ Hz, Fmoc), 5.12 (s, 1H, Man4-H-1), 5.00 (d, 1H, $J = 9.1$ Hz, GlcNAcl-H-1), 4.94 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.60 (b, 1H, GlcNAc2-H-1), 4.55 (d, 2H, $J = 8.4$ Hz, GlcNAc5,5′-H-1), 4.45 (d, 1H, $J = 8.0$ Hz, Gal6′-H-1), 4.35 (t, 1H, Fmoc), 4.25 (bd, 1H, Man3-H-2), 4.19 (bd, 1H, $J = 1.8$ Hz, Man4-H-2), 4.11 (bd, 1H, $J = 1.9$ Hz, Man4′-H-2), 2.72 (bd, 1H, Asn-βCH), 2.67 (dd, 1H, $J = 4.7$ Hz, 12.6 Hz, NeuAc7′-H-3eq), 2.52 (bdd, 1H, $J = 9.7$ Hz, 14.8 Hz, Asn-βCH), 2.06 (s, 6H, Ac × 2), 2.05, 2.03, 1.89 (each s, each 3H, Ac), 1.79 (dd, 1H, $J = 11.9$ Hz, 11.9 Hz, NeuAc7′-H-3ax).

General Purification Method for Exoglycosidase Digestion

After exoglycosidase digestion, the mixture is lyophilized, Fractionation by HPLC [YMC packed column D-ODS-5 S-5 120A, ϕ 20×250 mm; eluted with 50 mM ammonium acetate–CH_3CN, 3:2 (v/v); 4.0 ml/min; monitoring at 215 nm] affords oligosaccharide. The fraction containing the desired product is pooled and lyophilized. Removal of ammonium acetate is accomplished by HPLC (ODS column; ϕ 5×150 mm; H_2O, 100 ml, and then 25% CH_3CN, 200 ml, monitoring at 215 nm), affording a pure oligosaccharide.

General Procedure for N-Acetyl-β-D-glucosaminidase Digestion

To a solution of substrate (48 μmol) in HEPES buffer (50 nM, pH 6.0, 1.8 ml) containing bovine serum albumin (1 mg) is added N-acetyl-β-D-glucosaminidase (1.6 U in 50 mM HEPES buffer, 100 μl, pH 6.0), with incubation at 37°. After completion of the digestion (~1 day) as monitored by HPLC [ODS, ϕ 5×150 mm; 50 mM ammonium acetate–CH_3CN, 8:2 (v/v) monitoring at 215 nm], the mixture is lyophilized.

General Procedure for β-D-Mannosidase Digestion

To a solution of substrate (24 μmol) in HEPES buffer (50 mM, pH 6.0, 0.9 ml) containing bovine serum albumin (1 mg) is added β-D-mannosidase (0.5 U in 50 mM HEPES buffer, 50 μl, pH 6.0) and this solution is incubated at 37°. After completion of the digestion (~1 day) as monitored by HPLC [ODS, ϕ 5×150 mm, 50 mM ammonium acetate–CH_3CN, 8:2 (v/v) monitoring at 215 nm], the mixture is lyophilized.

General Procedure for α-D-Neuraminidase Digestion

To a solution of substrate (18.4 μmol) in HEPES buffer (50 mM, pH 6.0, 0.72 ml) containing bovine serum albumin (1 mg) is added α-D-neuraminidase (134 mU in 50 mM HEPES buffer, 50 μl, pH 6.0) and this solution is incubated at 37°. After completion of the digestion (~1 day) as monitored by HPLC [ODS, ϕ 5×150 mm, 50 mM ammonium acetate–CH_3CN, 8:2 (v/v) monitoring 215 nm], the mixture is lyophilized.

General Procedure for β-D-Galactosidase Digestion

To a solution of substrate (6.2 μmol) in HEPES buffer (50 mM, pH 6.0, 0.6 ml) containing bovine serum albumin (1 mg) is added β-D-galactosidase (180 mU in 50 mM HEPES buffer, 50 μl, pH 6.0) and this solution is incubated at 37°. After completion of the digestion (~30 h) as monitored

by HPLC [ODS, ϕ 5 × 150 mm, 50 mM ammonium acetate–CH_3CN, 8:2 (v/v) monitoring at 215 nm], the mixture is lyophilized.

*Compound **18***

^{1}H NMR δ 5.11 (s, 1H, Man4-H-1), 5.00 (d, 1H, $J = 9.7$ Hz, GlcNAc1-H-1), 4.95 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.45 (d, 1H, $J = 7.9$ Hz, Gal6′-H-1), 4.25 (bs, 1H, Man3-H-2), 4.12 (bdd, 1H, Man4′-H-2), 4.07 (bdd, 1H, Man4-H-2), 2.68 (dd, 2H, $J = 4.8$ Hz, 12.4 Hz, NeuAc7′-H-3eq), 1.72 (dd, 1H, $J = 12.4$ Hz, 12.4 Hz, Neu Ac7′-H-3ax).

*Compound **19***

^{1}H NMR δ 5.00 (d, 1H, $J = 9.4$ Hz, GlcNAc1-H-1), 4.94 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.64–4.53 (1H, GlcNAc2-H-1), 4.64–4.53 (1H, GlcNAc5′-H-1), 4.45 (d, 1H, $J = 8.0$ Hz, Gal6′-H-1), 4.28 (b, 1H, Man3-H-2), 4.11 (b, 1H, Man4′-H-2), 2.68 (dd, 1H, $J = 4.6$ Hz, 12.5 Hz, NeuAc7′-H-3eq), 1.72 (dd, 1H, $J = 12.5$ Hz, 12.5 Hz, NeuAc7′-H-3ax).

*Compound **40***

^{1}H NMR δ 5.14 (s, 1H, Man4-H-1), 5.00 (d, 1H, J = 9.4 Hz, GlcNAc1-H-1), 4.92 (s, 1H, Man4′-H-1), 4.78 (s, 1H, Man3-H-1), 4.44 (d, 1H, $J = 8.0$ Hz, Gal6-H-1), 4.35 (t, 1H, Fmoc), 4.25 (bd, 1H, Man3-H-2), 4.20 (bdd, 1H, Man4-H-2), 2.67 (dd, 1H, $J = 4.6$ Hz, 12.2 Hz, NeuAc7-H-3eq), 1.72 (dd, 1H, $J = 12.2$ Hz, 12.2 Hz, NeuAc7-H-3ax).

*Compound **41***

^{1}H NMR δ 5.14 (s, 1H, Man4-H-1), 5.00 (d, 1H, $J = 9.4$ Hz, GlcNAc1-H-1), 4.78 (s, 1H, Man3-H-1), 4.45 (d, 1H, $J = 8.0$ Hz, Gal6-H-1), 4.23 (bd, 1H, Man3-H-2), 4.20 (bd, 1H, Man4-H-2), 2.67 (dd, 1H, $J = 4.8$ Hz, 12.4 Hz, NeuAc7-H-3eq), 1.72 (dd, 1H, $J = 12.4$ Hz, 12.4 Hz, NeuAc7-H-3ax).

*Compound **28***

^{1}H NMR δ 5.12 (s, 1H, Man4-H-1), 4.99 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.92 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.58 (d, 1H, $J = 8.0$ Hz, GlcNAc2-H-1), 4.55 (d, 1H, $J = 8.4$ Hz, GlcNAc5,5′-H-1), 4.47 (d, 1H, $J = 7.8$ Hz, Gal6′-H-1), 4.24 (bd, 1H, $J = 1.9$ Hz Man3-H-2), 4.18 (bdd, 1H, $J = 1.4$ Hz, 3.3 Hz, Man4-H-2), 4.11 (bdd, 1H, $J = 1.4$ Hz, 3.5 Hz, Man4′-H-2).

*Compound **30***

^{1}H NMR δ 5.10 (s, 1H, Man4-H-1), 4.99 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.92 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1) 4.58 (d, 2H,

GlcNAc2,5′-H-1), 4.47 (d, 1H, $J = 8.0$ Hz, Gal6′-H-1), 4.24 (bd, 1H, $J = 1.9$ Hz, Man3-H-2), 4.11 (bs, 1H, Man4′-H-2), 4.07 (bs, 1H, Man4-H-2).

*Compound **31***

^{1}H NMR δ 5.10 (s, 1H, Man4-H-1), 4.99 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.55 (d, 2H, GlcNAc2,5′-H-1), 4.24 (bs, 1H, Man3-H-2), 4.10 (bs, 1H, Man4′-H-2), 4.06 (bs, 1H, $J = 1.3$ Hz, Man4-H-2).

*Compound **38***

^{1}H NMR δ 5.12 (s, 1H, Man4-H-1), 4.99 (d, 1H, $J = 9.4$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.57 (bd, 2H, GlcNAc2,5-H-1), 4.46 (d, 1H, $J = 7.5$ Hz, Gal6-H-1), 4.24 (bs, 1H, Man3-H-2), 4.19 (bs, 1H, Man4-H-2).

*Compound **45***

^{1}H NMR δ 5.11 (s, 1H, Man4-H-1), 4.99 (d, 1H, $J = 9.4$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.55 (d, 2H, GlcNAc2,5-H-1), 4.24 (bs, 1H, Man3-H-2), 4.18 (bs, 1H, Man4-H-2), 3.97 (dd, 1H, $J = 1.8$Hz, 3.3 Hz, Man4′-H-2).

*Compound **20***

^{1}H NMR δ 5.00 (d, 1H, $J = 9.9$ Hz, GlcNAc1-H-1), 4.92 (s, 1H, Man4′-H-1), 4.75 (s, 1H, Man3-H-1), 4.58 (d, 2H, $J = 7.5$ Hz, GlcNAc2,5′-H-1), 4.47 (d, 1H, $J = 7.8$ Hz, Gal6′-H-1), 4.10 (bd, 1H, Man4′-H-2), 4.07 (bs, 1H, Man3-H-2).

*Compound **21***

^{1}H NMR δ 4.99 (d, 1H, $J = 9.7$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.55 (d, 1H, $J = 8.1$ Hz, GlcNAc2,5′-H-1), 4.09 (bd, 1H, Man4′-H-2), 4.07 (s, 1H, Man3-H-2).

*Compound **22***

^{1}H NMR δ 5.00 (d, 1H, $J = 9.7$ Hz, GlcNAc1-H-1), 4.91 (d, 1H, $J = 1.6$ Hz, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.58 (d, 1H, $J = 7.8$ Hz, GlcNAc2-H-1), 4.07 (d, 1H, $J = 2.7$ Hz, Man3-H-2), 3.97 (dd, 1H, $J = 1.6$ Hz, 3.7 Hz, Man4′-H-2).

*Compound **42***

^{1}H NMR δ 5.12 (s, 1H, Man4-H-1), 4.99 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.77 (s, 1H, Man3-H-1), 4.57 (d, 2H, $J = 7.2$ Hz, GlcNAc2-H-1), 4.46 (d, 1H, $J = 7.8$ Hz, Gal6-H-1), 4.22 (bd, 1H, $J = 2.7$ Hz, Man3-H-2), 4.19 (b, 1H, Man4-H-2).

*Compound **43***

^{1}H NMR δ 5.12 (s, 1H, Man4-H-1), 4.99 (d, 1H, $J = 9.7$ Hz, GlcNAc1-H-1), 4.76 (s, 1H, Man3-H-1), 4.55 (d, 2H, $J = 8.6$ Hz, GlcNAc2,5-H-1), 4.22 (d, 1H, $J = 2.2$ Hz, Man3-H-2), 4.18 (bdd, 1H, $J = 1.3$ Hz, 3.3 Hz, Man4-H-2).

*Compound **44***

^{1}H NMR δ 5.11 (s, 1H, Man4-H-1), 4.99 (d, 1H, $J = 9.7$ Hz, GlcNAc1-H-1), 4.77 (s, 1H, Man3-H-1), 4.57 (d, 1H, $J = 6.5$ Hz, GlcNAc-H-1), 4.22 (d, 1H, $J = 3.0$ Hz, Man3-H-2), 4.07 (bdd, 1H, $J = 2.1$ Hz, Man4-H-2).

*Compound **34***

^{1}H NMR δ 5.11 (s, 1H, Man4-H-1), 4.99 (1H, d, $J = 9.9$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.55 (d, 3H, $J = 8.6$ Hz, GlcNAc2,5,5′-H-1), 4.24 (s, 1H, Man3-H-2), 4.18 (s, 1H, Man4-H-2), 4.10 (s, 1H, Man4′-H-2).

*Compound **35***

^{1}H NMR δ 5.10 (s, 1H, Man4-H-1), 4.99 (d, 1H, $J = 9.7$ Hz, GlcNAc1-H-1), 4.91 (bd, 1H, $J = 1.6$ Hz, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.24 (bs, 1H, Man3-H-2), 4.06 (dd, 1H, $J = 1.6$ Hz, 3.2 Hz, Man4-H-2), 3.97 (dd, 1H, $J = 1.6$ Hz, 3.5 Hz, Man4′-H-2).

General Procedure for De-N-9-fluorenylmethyl Group

To a solution of Fmoc substrate (1 μmol) in DMF (240 μl) is added morpholine (160 μl) and this mixture is stirred at room temperature. After finishing the reaction (~7–10 h), to this mixture is added ether (4.0 ml) and the precipitate is then collected. Purification of the residue by HPLC (ODS column; ϕ 5 × 150 mm; H_2O, 100 ml, and then 25% CH_3CN, 200 ml, with monitoring at 215 nm) affords Asn-oligosaccharide.

*Compound **17***

^{1}H NMR δ 5.13 (s, 1H, Man4-H-1), 5.07 (d, 1H, $J = 9.9$ Hz, GlcNAc1-H-1), 4.95 (s, 1H, Man4′-H-1), 4.78 (s, 1H, Man3-H-1), 4.62 (2H, GlcNAc2,5′-H-1), 4.56 (d, 1H, $J = 8.1$ Hz, GlcNAc5-H-1), 4.52

(d, 1H, $J = 7.8$ Hz, Gal6′-H-1), 4.25 (bs, 1H, Man3-H-2), 4.19 (bs, 1H, Man4-H-2), 4.12 (bs, 1H, Man4′-H-2), 2.68 (dd, 1H, $J = 4.6$ Hz, 12.4Hz, NeuAc7′-H-3eq), 1.72 (dd, 1H, $J = 12.1$ Hz, 12.1 Hz, NeuAc7′-H-3ax).

Compound **23**

^{1}H NMR δ 5.11 (s, 1H, Man4-H-1), 5.08 (d, 1H, J = 9.7 Hz, GlcNAc1-H-1), 4.95 (s, 1H, Man4′-H-1), 4.78 (s, 1H, Man3-H-1), 4.62 (d, 2H, GlcNAc2,5′-H-1), 4.45 (d, 1H, $J = 7.6$ Hz, Gal6′-H-1), 4.26 (bd, 1H, Man3-H-2), 4.12 (bd, 1H, Man4′-H-2), 4.08 (bdd, 1H, $J = 1.6$ Hz, 3.3 Hz, Man4-H-2), 2.68 (dd, 1H, $J = 4.1$ Hz, 12.1 Hz, NeuAc7′-H-3eq), 1.72 (dd, 1H, $J = 12.1$ Hz, 12.1 Hz, NeuAc7′-H-3ax).

Compound ***24***

^{1}H NMR δ 5.07 (d, 1H, $J = 9.4$ Hz, GlcNAc1-H-1), 4.94 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.61 (d, 1H, GlcNAc2-H-1), 4.59 (d, 1H, GlcNAc5′-H-1), 4.44 (d, 1H, $J = 7.8$ Hz, Gal6′-H-1), 4.10 (bs, 1H, Man4′-H-2), 4.07 (1H, Man3-H-2), 2.67 (dd, 1H, $J = 4.6$ Hz, 12.2 Hz, NeuAc7′-H-3eq), 1.71 (2H, dd, $J = 12.2$ Hz, 12.2 Hz, NeuAc7′-H-3ax).

Compound ***6***

^{1}H NMR δ 5.13 (s, 1H, Man4-H-1), 5.06 (d, 1H, $J = 9.9$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.61 (d, 1H, $J = 8.0$ Hz, GlcNAc2-H-1), 4.60 (d, 1H, $J = 8.0$ Hz, GlcNAc5-H-1), 4.55 (d, 1H, $J = 8.4$ Hz, GlcNAc5′-H-1), 4.44 (d, 1H, $J = 8.0$ Hz, Gal6-H-1), 4.24 (bd, 1H, Man3-H-2), 4.19 (bdd, 1H, $J = 1.3$ Hz, 3.2 Hz, Man4-H-2), 4.10 (bdd, 1H, $J = 1.4$ Hz, 3.2 Hz, Man4′-H-2), 2.66 (dd, 1H, $J = 4.6$ Hz, 12.4 Hz, NeuAc7-H-3eq), 1.71 (dd, 1H, $J = 12.4$ Hz, 12.4 Hz, NeuAc7-H-3ax).

Compound ***46***

^{1}H NMR δ 5.12 (s, 1H, Man4-H-1), 5.06 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.61 (d, 1H, GlcNAc2-H-1), 4.59 (d, 1H, GlcNAc5-H-1), 4.43 (d, 1H, $J = 8.0$ Hz, Gal6-H-1), 4.24 (bd, 1H, Man3-H-2), 4.18 (bdd, 1H, Man4-H-2), 2.66 (dd, 1H, $J = 4.6$ Hz, 12.6 Hz, NeuAc7-H-3eq), 1.70 (dd, 1H, $J = 12.6$ Hz, 12.6 Hz, NeuAc7-H-3ax).

Compound ***47***

^{1}H NMR δ 5.14 (s, 1H, Man4-H-1), 5.07 (d, 1H, $J = 9.4$ Hz, GlcNAc1-H-1), 4.78 (s, 1H, Man3-H-1), 4.61 (d, 1H, GlcNAc2-H-1), 4.60 (d, 1H, GlcNAc5-H-1), 4.44 (d, 1H, $J = 8.0$ Hz, Gal6-H-1), 4.23 (d, 1H, $J = 3.0$ Hz, Man3-H-2), 4.19 (bdd, 1H, $J = 1.3$ Hz, 2.9 Hz, Man4-H-2), 2.67 (dd, 1H, $J = 4.6$ Hz, 12.7 Hz, NeuAc7-H-3eq), 1.71 (dd, 1H, $J = 12.7$ Hz, 12.7 Hz, NeuAc7-H-3ax).

Compound ***29***

^{1}H NMR δ 5.11 (s, 1H, Man4-H-1), 5.06 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.92 (s, 1H, Man4′-H-1), 4.75 (s, 1H, Man3-H-1), 4.61 (d, 1H, $J = 7.5$ Hz, GlcNAc2-H-1), 4.57, 4.55 (each d, each 1H, $J = 7.5$ Hz, GlcNAc5,5′-H-1), 4.46 (d, 1H, $J = 7.3$ Hz, Gal6′-H-1), 4.23 (bs, 1H, Man3-H-2), 4.18 (bs, 1H, Man4-H-2), 4.10 (bs, 1H, Man4′-H-2).

Compound ***7***

^{1}H NMR δ 5.11 (s, 1H, Man4-H-1), 5.06 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.60 (d, 1H, GlcNAc2-H-1), 4.57, 4.55 (each d, each 1H, GlcNAc5,5′-H-1), 4.46 (d, 1H, $J = 7.8$ Hz, Gal6-H-1), 4.28 (s, 1H, Man3-H-2), 4.18 (s, 1H, Man4-H-2), 4.10 (s, 1H, Man4′-H-2).

Compound ***32***

^{1}H NMR δ 5.10 (s, 1H, Man4-H-1), 5.07 (d, 1H, $J = 9.4$ Hz, GlcNAc1-H-1), 4.92 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.61 (d, 1H, $J = 7.8$ Hz, GlcNAc2-H-1), 4.57 (d, 1H, $J = 7.8$ Hz, GlcNAc5′-H-1), 4.47 (d, 1H, $J = 7.8$ Hz, Gal6′-H-1), 4.24 (d, 1H, $J = 2.3$ Hz, Man3-H-2), 4.10 (bd, 1H, Man4′-H2), 4.06 (bd, 1H, Man4-H-2).

Compound ***33***

^{1}H NMR δ 5.09 (s, 1H, Man4-H-1), 5.06 (d, 1H, $J = 9.8$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.61 (d, 1H, GlcNAc2-H-1), 4.54 (d, 1H, GlcNAc5′-H-1), 4.24 (s, 1H, Man3-H-2), 4.10 (bd, 1H, Man4′-H2), 4.06 (bs, 1H, Man4-H-2).

Compound ***39***

^{1}H NMR δ 5.11 (s, 1H, Man4-H-1), 5.07 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.61 (d, 1H,

GlcNAc2-H-1), 4.57 (d, 1H, GlcNAc5-H-1), 4.46 (d, 1H, Gal6-H-1), 4.24 (s, 1H, Man3-H-2), 4.18 (bs, 1H, Man4-H-2).

Compound ***51***

^{1}H NMR δ 5.11 (s, 1H, Man4-H-1), 5.06 (d, 1H, $J = 9.9$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.60 (d, 1H, $J = 7.9$ Hz, GlcNAc2-H-1), 4.54 (d, 1H, $J = 7.9$ Hz, GlcNAc5-H-1), 4.24 (s, 1H, Man3-H-2), 4.18 (dd, 1H, $J = 1.6$ Hz, 1.6 Hz, Man4-H-2), 3.96 (1H, dd, $J = 1.6$ Hz, 1.6 Hz, Man4′-H-2).

Compound ***25***

^{1}H NMR δ 5.07 (d, 1H, $J = 9.7$ Hz, GlcNAc1-H-1), 4.92 (s, 1H, Man4′-H-1), 4.75 (s, 1H, Man3-H-1), 4.62 (d, 1H, GlcNAc2-H-1), 4.58 (d, 1H, GlcNAc5′-H-1), 4.09 (s, 1H, Man4′-H-2), 4.08 (s, 1H, Man3-H-2).

Compound ***26***

^{1}H NMR δ 5.07 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.62 (d, 1H, GlcNAc2-H-1), 4.55 (d, 1H, GlcNAc5′-H-1), 4.10 (bs, 1H, Man4′-H2), 4.07 (s, 1H, Man3-H-2).

Compound ***27***

^{1}H NMR δ 5.07 (d, 1H, $J = 9.5$ Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.76 (s, 1H, Man3-H-1), 4.62 (d, 1H, $J = 7.8$ Hz, GlcNAc2-H-1), 4.08 (d, 1H, $J = 2.9$ Hz, Man3-H-2), 3.97(bs, 1H, Man4′-H-2).

Compound ***48***

^{1}H NMR δ 5.11 (s, 1H, Man4-H-1), 5.06 (d, 1H, J = 9.8 Hz, GlcNAc1-H-1), 4.77 (s, 1H, Man3-H-1), 4.61 (d, 1H, GlcNAc2-H-1), 4.57 (d, 1H, GlcNAc5-H-1), 4.46 (d, 1H, $J = 7.8$ Hz, Gal6-H-1), 4.22 (bs, 1H, Man3-H-2), 4.18 (bs, 1H, Man4-H-2).

Compound ***49***

^{1}H NMR δ 5.12 (s, 1H, Man4-H-1), 5.07 (d, 1H, J = 9.7 Hz, GlcNAc1-H-1), 4.77 (s, 1H, Man3-H-1), 4.61 (d, 1H, GlcNAc2-H-1), 4.54 (d, 1H, GlcNAc5-H-1), 4.22 (d, 1H, $J = 2.5$ Hz, Man3-H-2), 4.18 (dd, 1H, $J = 1.4$ Hz, 3.0 Hz, Man4-H-2).

Compound ***50***

^{1}H NMR δ 5.10 (s, 1H, Man4-H-1), 5.06 (d, 1H, J = 9.5 Hz, GlcNAc1-H-1), 4.77 (s, 1H, Man3-H-1), 4.61 (d, 1H, J = 7.3 Hz, GlcNAc2-H-1), 4.22 (d, 1H, J = 2.4 Hz, Man3-H-2), 4.07 (dd, 1H, J = 1.6 Hz, 3.0 Hz, Man4-H-2).

Compound ***36***

^{1}H NMR δ 5.11 (s, 1H, Man4-H-1), 5.07 (d, 1H, J = 10.0 Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.77 (s, 1H, Man3-H-1), 4.61 (d, 1H, J = 6.8 Hz, GlcNAc2-H-1), 4.55 (d, 2H, GlcNAc5,5′-H-1), 4.24 (bs, 1H, Man3-H-2), 4.18 (bs, 1H, Man4-H-2), 4.10 (bs, 1H, Man4′-H-2).

Compound ***37***

^{1}H NMR δ 5.10 (s, 1H, Man4-H-1), 5.07 (d, 1H, J = 9.7 Hz, GlcNAc1-H-1), 4.91 (s, 1H, Man4′-H-1), 4.78 (s, 1H, Man3-H-1), 4.61 (d, 1H, J = 8.0 Hz, GlcNAc2-H-1), 4.25 (bs, 1H, Man3-H-2), 4.06 (bs, 1H, Man4-H-2), 3.97 (bs, 1H, Man4′-H-2).

[6] Enzymatic Synthesis of Neoglycoconjugates by Transglycosylation with Endo-β-*N*-acetylglucosaminidase A

By KAORU TAKEGAWA and JIAN-QIANG FAN

Introduction

The oligosaccharide moieties of glycoproteins have been shown to play important roles in biological processes such as cellular recognition, lectin binding, viral infection, and substrate–receptor recognition.[1] In general, naturally occurring glycoproteins have a high degree of heterogeneity in their oligosaccharide moiety, and it is difficult to clarify the biological roles of individual oligosaccharides. Therefore, to effectively elucidate the significance and function of each oligosaccharide, efficient methods for the synthesis and construction of a neoglycoprotein with a single type of carbohydrate moiety are required. Several strategies for the attachment of naturally occurring N-linked oligosaccharides to proteins have been described. These include the chemical addition of N-linked oligosaccharides to

[1] A. Varki, *Glycobiology* **3,** 97 (1993).

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